Hepatitis C Virus Infection and Vaccine Development

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In the twenty-seven years since the discovery of hepatitis C virus (HCV) the majority of individuals exposed to HCV establish a persistent infection, which is a leading cause of chronic liver disease, cirrhosis and hepatocellular carcinoma. In developed nations, the cure rates of HCV infection could be over 90% with direct-acting antiviral (DAA) regimens, which has made the great progress in global eradication. However, the cost of these treatments is so expensive that the patients in developing nations, where the disease burden is the most severe, could not afford it, which highly restricted its access. Additionally, the largely asymptomatic nature of infection facilitates continued transmission in risk groups due to limited surveillance. Consequently a protective vaccine and likely emergence of drug-resistant viral variants call for further studies of HCV biology. In the current review, the development and the progress of preventive and therapeutic vaccines against the HCV have been reviewed in the context of peptide vaccines, recombinant protein vaccines, HCV-like particle, DNA vaccines and viral vectors expressing HCV genes. (J CLIN EXP HEPATOL 2018;8:195–204)

Hepatitis C virus (HCV) is a positive-strand RNA virus, which was identified by molecular cloning in 1989 and classified into seven genotypes within its own genus.¹ Over 160 million individuals have been infected by HCV worldwide; wherein, approximately 80% of cases could lead to chronic liver disease.^{2–4} The open reading frame of HCV encodes for a large polyprotein with three structural proteins, Core (C), E1 and E2 that are linked to the nonstructural proteins NS1, NS2, NS3, NS5A and NS5B via the presumed viroporin p7 (Table 1).^{5,6} The structural proteins form the viral particle, while the nonstructural proteins are involved in replication and maturation of the virus particle.⁷ HCV infection is characterized by a high propensity for development of life-long viral persistence.^{8,9} Only one in five acute infections could be eradicated spontaneously, normally within the first six months after infection.^{10,11} During initial time for acute HCV infections, clinical symptoms are mild or even absent. For that reason acute HCV infections are often not recognized. However, when acute HCV infection develops into a persistent infection, the majority of the patients would turn into chronic hepatitis

and over decades the virus causes subtle but cumulative hepatic damage. Ultimately the patients may develop either to cirrhosis, decompensating liver congestion or hepatocellular carcinoma. To give a sense of the impact of HCV infection on the health care system, it has been calculated that 27% of the cases of cirrhosis can be accounted for HCV worldwide, and population-based studies in the United States indicated that 40% of chronic liver disease is HCV related.^{12,13} Overall, persistent HCV infection would be responsible for 3 million deaths each year.¹³

If there is HCV infection, the first responding system contains proinflammatory cytokines and a cellular component. In addition to resisting infection, innate immunity is also involved in provoking adaptive immunity.¹⁴ Three arms of innate immunity that identify HCV infection as a threat are (Figure 1): (1) RIG-I-like receptors (RLRs); (2) TLRs; and (3) nucleotide oligomerization domain (NOD)-like receptors (NLRs).¹⁵ After HCV recognition, various downstream signals would be sent to induce the production of various cytokines, such as interleukins (IL) and IFNs. They would create an antiviral state for uninfected cells, decrease HCV replication in infected cells, and link innate immunity to adaptive immunity.¹⁴ Humoral responses to HCV infection include B cell activation and production of antibodies that are primarily low titers of IgG1 and appear late in the course of the disease.^{16,17} Antibodies then can be detected after 7–10 wk of infection; however, viral RNA can be only detected 1–3 wk after infection.¹⁸ Although a few antibodies toward E1 glycoprotein and NS proteins have already been found, the main target for antibodies is the surface E2 glycoprotein.^{17,19,20} Antibodies against NS proteins were found earlier in the disease course with a greater magnitude. Nevertheless, E2 glycoprotein is still considered to be

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Abbreviations: DAA: direct-acting antiviral; HCV: hepatitis C virus; HIV: human immunodeficiency virus

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Table 1 Functions of HCV Genomic Proteins.

Protein	Hepatitis C virus gene function
NS4B	Replication complex
NS3	Helicase activity
NS5B	Formation of replication complex
p7	Processing of polyprotein, viral assembly
NS2/NS3	Protease activity
NS3/NS4A	Serine protease activity
E1 and E2	Glycoproteins of envelope

the main target for antibodies. In the recent studies, patients infected by HCV would have an elevated level of activated B cells, and the ones with defects in antibodies experience rapid disease progression, which emphasizes the role of humoral immunity in HCV.

Cell immunity for HCV is composed of two arms: CD4⁺ and CD8⁺ T cells that are detectable in peripheral blood or the liver several weeks post-infection.²¹ These T cells could directly kill infected cells or produce some soluble factors that could clear the virus in a non-cytolytic way. These cells could also directly damage the liver or attract non-specific inflammatory cells and finally lead to the acceleration of liver inflammation.²² Based on the recognition of HCV through the major histocompatibility complex (MHC) class II, CD4⁺ T cells (T helper) mainly produce IFN- γ that is correlated with the decreasing HCV titer and transaminase levels.²¹ These cells could also produce IL-2 that would do a great help to the production of CD8⁺ (cytotoxic) T cells. Moreover, they can enhance B cell production. T helper actions are essential for spontaneous recovery during acute HCV.²³ It has been shown that T helper responses could not be observed in patients who are suffering from chronic disease.²⁴ CD8⁺ T cells recognize HCV-infected cells through MHC class I and then

Figure 1 The hepatitis C virus persistence: how to evade the immune system.

lead to lysis of infected cells.²⁵ The role of MHC class I in HCV recognition indicates that spontaneous clearance of HCV have some associations with the presence of some MHC class I molecules.²⁶

Despite the theory and method in the prevention and treatment of HCV infection has made great progress in the world, the understanding of the molecular immune mechanism of HCV is not thoroughly explained, combining with the lack of stability of HCV cell culture models as well as the ideal experimental animal models. All these disadvantages above would directly restricted the effective prevention and treatment of HCV. This review is mainly aimed at the vaccine development, antiviral immune responses in the control of HCV infection and HCV infection models integrating the HCV vaccine research.

THE POSSIBILITY OF HCV VACCINE DEVELOPMENT

There is compelling evidence that the spontaneous resolution of HCV infection would protect against persistence upon re-exposure to the virus in 30% of cases. Rechallenge of immune chimpanzees with HCV results in viremia, but much shorter duration and peak magnitude than in primary infections.²⁷ Most importantly, the rate of persistence is much lower in second HCV infections compared with that in the first HCV infections, even when the rechallenge was undertaken years later.²⁷ A protective effect of a prior resolved infection is also apparent in humans; prospective studies in injection drug users revealed that 80% of primary HCV infections persist, while only 20% of secondary infections in those who cleared an earlier infection.^{28,29} A T-cell vaccine for HCV has been a realistic goal since a significant number of individuals spontaneously clear HCV by the establishment of an appropriate immune response. And there would be protective immunological memory appearing to against HCV in chimpanzees and humans, where secondary infection is associated with reductions in peak and duration of viremia, hepatic inflammation, and an increased rate of viral clearance.^{27,29,30} Comparative analyses of individuals with distinct clinical outcomes have been carried out by several groups, and there is now some consensus on the immune response required to prevent persistence of HCV, but there is no single correlate of protection.^{21,31} During acute infection, a broad, strong and persistent HCV-specific T-cell response is required for spontaneous clearance.^{32–34} T-cell dysfunction is a hallmark of chronic viral infection, including T-cell exhaustion, inhibition by regulatory T-cells, and T-cell escape. Emerging evidence supports the protective role of virus neutralizing antibodies well, and the ability of the B cell response of modifying the course of infection.³⁵ B cells and rapid induction of cross-reactive neutralizing antibodies (nAbs) responses play an active role in the spontaneous recovery of HCV infection,^{36–38}

where nAbs target epitopes within the HCV envelope glycoproteins E1 and E2, or the E1E2 heterodimer. Studies demonstrated that most of the identified nAbs target regions were found within E2,³⁹ including the “hyper-variable region 1”.³⁵ However, it was previously suggested that neutralizing antibodies identified in chronic HCV patients are not able to control chronic HCV infection. The neutralizing antibody response of the host lags behind the fast emerging HCV envelope glycoprotein sequences of the quasispecies population.⁴⁰ The IL28B gene encodes the cytokine IFN-lambda3 (IFN- λ 3), which belongs to the Type III IFN family (IFN- λ). IFN- λ could be induced rapidly after HCV infection and has antiviral activity against HCV.⁴¹ The understanding the mechanism of IL28B polymorphism is still limited. IL28B polymorphism appears to affect IFN- λ 3 expression, with the unfavorable genotypes resulting in the reduced IFN- λ 3 expression. Patients with the unfavorable genotypes also had a lower induction of innate immunity genes, suggesting that IL28B polymorphism may regulate innate immune functions.⁴²

LACK OF AN APPROPRIATE HCV MODEL

Other than humans, chimpanzees and a nonrodent small mammal named *Tupaia belangeri*, known as the tree shrew, are naturally susceptible to HCV infection.^{43,44} In addition to the mentioned animal models above, much effort has been made to introduce genetically-manipulated animals, preferably mice or rats, for the further study of HCV pathogenesis and vaccine design.^{45–48} The results of chimpanzee studies are highly variable and difficult to interpret because of the genetic variability appearing between animals and small sample sizes. Approximately 30–40% of chimpanzees develop chronic HCV infection, whereas 85% of infected humans develop chronic HCV.^{48,49} The high cost of acquiring and maintaining the animals, their limited availability, and ethical considerations are major drawbacks to the use of chimpanzees for HCV animal models.⁴² For tree shrews, the infection rate is low and the viremia is rarely sustained; however, they can also develop chronic hepatitis in some cases.^{50,51} But like the chimpanzees, there are also some disadvantages that would limit their use for the study of HCV such as the limited availability of tree shrews, their high housing costs, and the lack of tupaia specific reagents (used to assess HCV-host interactions).⁴³

HCV can replicate in lymphoid cells or PBMCs but only for limited periods of time and very low viral loads.⁵² The invention of HCV pseudo-particles (HCVpp) expressing unmodified E1E2 glycoproteins has provided us a way for more thorough studies toward HCV specific antibodies,⁵³ although the structure and neutralization of HCVpp are significantly different from natural HCV.³⁹ The first successful tissue model of HCV infection was discovered

in 2005 using the HCV/JFH1 cell culture system. The whole genome of the JFH1 virus was separated from a Japanese patient with fulminant hepatitis; it was then multiplied with PCR, cloned, and named JFH1. The JFH1 virus is of the 2a genotype and has a full-length HCV genome. This culturing system efficiently maintains the replication of HCV in human hepatoma cell line Huh7 and produces fair titers of cell-cultured derived HCV particles (HCVcc) with natural infectious properties. This platform was further broadened to include HCV subtype 1a strain H77-S, which is more commonly related to HCV complications, such as HCC and cirrhosis, and more resistant to IFN therapy than HCV genotype 2.⁵⁴ Recently, the hFLSCs have been successfully applied to efficient culture blood-borne hepatitis C virus in our group.⁵⁵ This model is expected to provide a powerful tool for exploring the process and the mechanism of bbHCV infection at the cellular level, and evaluating the treatment and preventive strategies of bbHCV infection.

HCV GENETIC VARIABILITY

Studies suggest that HCV first appeared over 1000 years ago and then evolved into seven distinct genotypes and more than 100 subtypes in different environments.^{3,56} HCV mutates at a rate of nearly one nucleotide per replication cycle. This is due in part to the lack of proofreading activity of NS5B RNA dependent polymerase that would cause an error rate of 10^{-3} to 10^{-5} per nucleotide per replication cycle.⁵⁷ These frequent mutations, in combination with a short viral half-life and rapid turnover (10^{10} – 10^{12} virions per day), lead to a high genetic variability which would cause the presence of distinct but closely related HCV variants (known as quasispecies) in one infected individual. E1 and E2 glycoproteins show the highest variability among all HCV proteins. It is estimated that HCV is 10 times more variable than the human immunodeficiency virus (HIV), and it do really pose a significant challenge for successful vaccine development.⁵⁸

PREVENTIVE AND THERAPEUTIC VACCINES

The potential risks of using attenuated HCV viral particles for vaccine production have resulted in the use of HCV genes and proteins formulated in novel vaccine modalities (Figure 2).⁵⁹ Neutralizing antibodies to highly conserved conformational and linear epitopes have been identified.³⁵ E2 crystal structure⁶⁰ has provided an essential framework to delineate the molecular interactions with CD81 and broadly neutralizing antibodies, which confirmed many of the salient points concluded from earlier receptor and antibody epitope mapping experiments.⁶¹ Different strategies have been developed to enhance the immunogenicity of multi-epitope DNA and peptide vaccines. Among these strategies: (1) using the proper adjuvant, where combining

vectored vaccines with protein antigen as adjuvant represent a valuable vaccine platform for infectious diseases where both T and B cells are crucial for protection^{62,63}; (2) improvement of delivery, where in vivo electroporation (EP), induce an efficient uptake of DNA by cells, significantly enhance T-cell responses, and increase expression of desirable gene⁶⁴; and (3) application of different vaccination regimens as DNA prime-peptide boost immunization regimen.⁶⁵ Current approaches for the vaccine against HCV are the recombinant of E1 and E2 proteins, synthetic peptides, virus-like particles (VLPs), recombinant non-pathogenic live vectors, DNA vaccines, dendritic cells, and primeboost strategies.^{66,67} A Swedish study evaluated therapeutic vaccination in standard of care therapy. The plasmid DNA encoding NS3/NS4A under the control of cytomegalovirus (CMV) immediate-early promoter was analyzed in a clinical Phase I/II a trial for treatment of naive HCV genotype 1 patients. The therapeutic outcome was proved to be safe and vaccinations significantly improved responses to HCV NS3.⁶⁸ The clinical evaluation of DNA vaccines, CIGB-230 containing core/E1/E2, was proved to enhance the immune response in non-responders to treatment with IFN plus ribavirin in phase one clinical trial.⁶⁹ In addition, therapeutic vaccines like viral-vector-based vaccine TG4040 proved to induce HCV-specific cellular immune responses, and reduced viral load in chronic HCV infected patients in phase I clinical trial.⁷⁰

RECOMBINANT PROTEIN VACCINES

Recombinant protein vaccines are developed by isolating the gene(s) encoding from the corresponding protein and cloning it/them in bacteria, yeast, or mammalian cells. This approach is based on the theory that an efficient number of viral epitopes can induce enough immune responses to develop protective immunity, generally including antibodies and CD4⁺ T cell responses. The advantages of recombinant protein vaccines are that they do not contain the pathogen or its genetic material and do not need organism culture.

In 1994, a recombinant heterodimeric E1E2 vaccine was tested on seven chimpanzees. Among those seven chimpanzees, sterilizing immunity against a homologous HCV strain appeared in five.⁷¹ Puig et al. vaccinated two chimpanzees, one naïve and the other one recovered from acute HCV infection, with recombinant HCV E1E2 glycoproteins.⁷² High antibody titers to E1E2 were observed due to strong T cell proliferative responses. After the interaction with HCV, viremia was delayed in both vaccinated animals compared to the non-immunized animals. Many other studies evaluating the efficacy of recombinant HCV vaccines in animals, especially chimpanzees, have achieved similar results, which paved the way for recombinant HCV vaccine trials in humans.^{73–75} The first prophylactic

Figure 2 Potential HCV vaccines in clinical phase development. The vaccines are based on either prophylactic or therapeutic usage in phase I, phase I/II or phase II development (no HCV-specific vaccine has reached phase III development yet). The biological component(s) of the vaccine is listed on top of the arrow. Sponsor or company conducting the trial is listed at the end of arrow along with clinical ID number (<http://www.clinicaltrials.gov>).

HCV vaccine tested in humans was T2S-918/InnoVac-C, a C-terminally shortened recombinant E1 protein with aluminum hydroxide (alum) adjuvant. This vaccine provoked a higher antibody response against E1 in healthy volunteers than in the patients who were suffering from persistent HCV infection. Studies on this vaccine, however, were ceased in 2007.⁷⁶ In 2011, Verstrepen et al. vaccinated four chimpanzees with either genotype 1b E1 or E2 recombinant with alum adjuvant and antibody responses were observed in all subjects.⁷⁷ Only antibodies against E1, however, were shown to neutralize HCV pseudo-particles. Another therapeutic vaccine, GI-5005, is based on recombinant core and NS3 proteins of HCV produced in yeast cells (*Saccharomyces cerevisiae*). Studies of in vitro and in vivo models demonstrated the robust immunogenicity of GI5005. Moreover, GI-5005 was evaluated in a phase I b clinical trial and displayed efficacy in patients with chronic HCV infection.⁷⁸ GI5005 and the standard

therapy [pegylated (PEG)-IFN/ribavirin] were evaluated in more than 250 chronic HCV-1 patients in a phase II, placebo-controlled trial. Improved early virological responses were observed in all treated naïve patients. The increase in sustained virological response rates were also observed in prior non-responder patients.⁷⁹ HCV E1E2 vaccine adjuvanted with MF59 acquired a higher seroconversion rate in the 20 μ g group and an anamnestic antibody response occurred following the 3rd immunization.⁸⁰

PEPTIDE VACCINES

Peptide vaccines could induce HCV specific T cell responses by presenting vaccine peptide to the T-cell receptor via HLA molecules. Therefore, they are HLA-specific and they could target only a selected subset of epitope sequences within the HCV genome. The high rate

of genetic variation in viruses within populations and geographic regions limits the universal usage of these vaccines. They often contain multiple epitopes in order to induce broader T cell responses. Unfortunately, some peptides may induce the production of T regulatory cells or immune tolerance.⁸¹ Intercell (Intercell AG, Vienna, Austria) developed a peptide vaccine, IC41, which consists of five synthetic peptides derived from conserved regions of core, NS3, and NS4 proteins of HCV genotypes 1 and 2 with a poly-L-arginine adjuvant. IC41 vaccination could induce few interferon producing cells and dose-dependent T cell immune responses. A team in Japan also worked on HCV peptide vaccines and published their corresponding findings in two papers.⁸² The first study was based on 12 HCV patients (genotype 1b) who had previously failed in standard IFN-based therapy. The study mainly assessed the functionality of a “personalized” vaccine containing four CD8⁺ A24 peptides that were combined with Freund's adjuvant. After the first vaccination, the rest of the vaccinations were carried out with specific peptides that produced responses in each participant. Although most patients developed peptidespecific T cell responses after the seventh injection, the authors observed a dose-dependent decrease in serum alanine aminotransferase (ALT) and HCV RNA levels from five and three patients, respectively.⁸² In the second study, another peptide vaccine composed of HCV core region (C35-44) peptides within ISA51 was found to be safe and well tolerated in a phase I trial on 25 HCV non-responder patients.⁸³ A phase I, dose-escalation, placebo-controlled randomized control trial was performed to assess a virosome-based peptide vaccine containing NS3 peptides in 30 healthy participants; however, there have been no results so far (ClinicalTrials.gov Identifier: [NCT00445419](https://clinicaltrials.gov/ct2/show/study?term=NCT00445419)).

CELL CULTURE BASED VACCINES

The development of an infectious cell culture system for HCV in 2005^{84,85} has aided much toward HCV vaccine research.⁸⁶ The choice of the cell culture is controlled by the permissiveness of HCV replication and the compliance of WHO cell cultures criteria. For this purpose, the choice of virus strains should base on the use of multiple strains with conserved epitopes from part or all of the HCV envelope region. The conserved epitopes could induce both neutralizing Ab and multispecific cellular immune responses implicating both CD4⁺ and CD8⁺ cells. A system for cell culture toward infectious HCV particles (HCVcc) has recently been established. Retroviral HCV pseudotypes (HCVpp) and recombinant cell culture-derived HCV (HCVcc) have been successfully used to study viral entry and antibody-mediated neutralization.⁸⁷ In vitro, HCV was proven to be controlled by antibody mediated neutralization targeting viral envelope.⁸⁸ Recently, experimental studies in mice demonstrated that immunization with

cell-culture-derived HCV could elicit HCV neutralizing antibodies and thus provide new insight for HCV vaccine production.^{89,90}

DNA VACCINES

One of the latest versions of vaccine is the immunization method based on DNA.⁹¹ DNA vaccines have shown superiority effects compared with conventional vaccines, such as recombinant protein based on live weakened viruses.⁹² DNA immunization advantages include feasibility of production, DNA manipulating simplicity and immune responses resulting from different origins such as T helper cell and cytotoxic T lymphocyte (CTL), and antibody responses.⁹¹ Also, DNA vaccines are suitable for sequential vaccinations since their function is not influenced by pre-existing antibody titers to the vector.⁹³

DNA-based vaccines are inferior to the traditional vaccines such as subunit vaccines since the intensity of the immune responses induced by DNA vaccines has been relatively weak, therefore researchers are tried to develop new technique like codelivery of novel cytokine IL-2, IL-7, IL-12, IL-15 and IL-18 adjuvants to circumvent this restriction.⁹⁴ HCV identification is indeed the most considerable development in viral disease recently. Because of the clinical significance of the disease, the development of new therapeutic strategies mainly focused on the study of molecular properties of the virus. An efficient HCV vaccine should stimulate the different aspects of the immune response such as broad humoral, T helper and CTL responses. Since the HCV genome demonstrates high heterogeneity and mutagenicity, generating prophylactic or therapeutic vaccine for HCV is still an unsolved problem. Previous studies have illustrated that the cellular immune responses might be essential for an efficient vaccine. New vaccine candidates, including DNA, peptide, recombinant protein and vector-based vaccines have demonstrated many advantages and lately have been put onto phase I/II human clinical trials. Some of these strategies could provide an acceptable antiviral immunity in healthy volunteers as well as the infected patients, but examining their effectiveness in infected or risk populations is still a challenge.

VIRUS VECTOR VACCINES

Viral vectors with the ability of expressing foreign antigens are the effective tool to induce T cell immunity and are promising for the induction of strong humoral responses against infectious diseases. Adenovirus, MVA, alphavirus or paramyxovirus vectors are examples for developing HCV vaccine.⁸⁶ Adenovirus (Ad) is considered to be one of the most potent vectors for eliciting CD8⁺ T cell and antibody responses in humans.⁹⁵ It was well recognized that combining adenovirus vector with protein antigen

can induce the production of strong antibody and T cell responses in experimental animals that surpass immune responses achieved by either vaccine alone.⁶² However, adenovirus immunogenicity may be unachievable due to anti-Ad preexisting immunity, since preexisting high-titer anti-vector Nabs may interfere with the immunological potency of such vaccines.⁹⁶ Chimpanzee adenoviruses were shown to be safe, highly immunogenic in humans, and insensitive to human adenovirus (HAdV) preexisting immunity.⁹⁷ A recent study demonstrated that adenoviral vectors with HCV nonstructural proteins expressed induced protective T cell responses in chimpanzees and were immunogenic in healthy volunteers.⁶² Furthermore, replication-defective, recombinant Sindbis virus vector with the express of the gene for HCV glycoproteins E2 and E1 was shown to induce effective humoral and cellular responses against HCV in vaccinated mice.⁶³ On the other hand, virus-like particles (VLPs) are attractive vectors for gene delivery as they could mimic the properties of native virions and they are safe and easily manufactured.³⁴

DENDRITIC CELL-BASED VACCINATION STRATEGIES

Recently, dendritic cell (DC)-based vaccines against HCV have been developed by a lot of researchers. Infusion of ex vivo stimulated DCs loaded with HCV antigens has been reported.⁹⁸ Induction of T cell mediated immune responses by DC vaccination is highly based on efficient antigen loading of the DCs.⁹⁹ An experimental study was conducted in mice to evaluate the efficacy of immunization with the NS5a-loaded DCs compared with that of plasmid encoding NS5a and NS5a protein. Vaccination with NS5a mRNA-transfected DCs or NS5a protein-pulsed DCs, induced stronger CD4⁺ and CD8⁺ T-cell responses significantly in comparison to vaccination with NS5a DNA-transfected DCs, plasmid encoding NS5 or rNS5a protein formulated with alum.⁹⁹ A recent experimental study in mice also showed that multi-epitope-based HCV vaccine targeting DCs offers an effective approach to inducing a broad immune response and viral clearance in chronic, HCV-infected patients.¹⁰⁰ As for this type of vaccines, no active human clinical trial has been reported.⁸⁶

EXPERT COMMENTARY

The Okairos/GSK vaccine is the first to enter phase I/II trials to prevent persistent HCV infection. The study is groundbreaking for vaccine science because it will test the very novel hypothesis that a vaccine designed for prime T cell immunity alone can prevent a serious human viral disease. Whether a reduction in the rate of HCV persistence, but not necessarily infection, is a viable endpoint for

a vaccine clinical trial will also be established. The vaccine is being assessed in individuals who have no evidence of prior HCV infection, but are still at risk of infection for the use of needle associated with intravenous drug use (see ClinicalTrials.gov [NCT01436357](https://clinicaltrials.gov/ct2/show/study/NCT01436357) for details). However, preventing reinfection after DAA cure of chronic hepatitis C may be the most pragmatic and targeted use of a preventive HCV vaccine. During chronic infection, CD8⁺ T cells are highly localized to the liver, functionally exhausted and/or could target escaped epitopes. CD4⁺ T cells are difficult to be detected in blood or liver during persistent HCV infection. Loss of antigen-specific CD4⁺ T cell activity occurs during the acute phase of infection and has not yet been explained. There may be multiple layers of negative regulation toward inhibitory signaling coming from receptors like PD-1 and possibly regulatory T cell activity.¹⁰¹ Whether these defects are restored after the cure of chronic hepatitis C with DAA is one of the most pressing questions when antiviral therapy or vaccines are considered to control HCV transmission. Two recent studies of CD8⁺ T cell immunity after DAA cure of chronic hepatitis C have addressed this question well. A comparison of all CD8⁺ T cell responses in DAA treated humans revealed enhanced antigen-driven proliferation after successful therapy, especially for those populations targeting invariant HCV epitopes.¹⁰² Detailed longitudinal study of two patients before and after cure documented showed that the CD127 expression on CD8⁺ T cells targeting an invariant epitope has increased. PD-1 expression decreased slightly, suggesting at least partial recovery of circulating CD8⁺ T cells from exhaustion.¹⁰² Another recent study assessed recovery of CD8⁺ T cells in a chimpanzee recovering from chronic hepatitis C directly and protection from re-infection. Intrahepatic CD8⁺ T cells targeting intact epitopes retained an exhausted phenotype characterized by high PD-1 and low CD127 for 2 years after cure. They did not expand in liver after rechallenge with HCV. Transient control of the second infection was associated with the expansion of CD8⁺ T cells targeting class I epitopes that were prone to escape. Persistence was kinetically linked to the rapid appearance of escape mutations in these epitopes. Because the impact of DAA therapy on phenotype and effector function was studied in very few CD8⁺ T cell populations selected from a small number of humans and chimpanzees. The vaccine need of preventing reinfection remains uncertain.

It is not yet known if infections that occur after DAA cure will resolve at the same high rate as primary HCV infections. Success will depend on the ability of the vaccine to restore potentially exhausted CD8⁺ T cell responses against epitopes that are not prone to escape, and to broaden the response to new epitopes not targeted during HCV infection. Under these circumstances, the distinction between preventive and therapeutic vaccination may begin to blur for HCV infection.

AUTHORS' CONTRIBUTIONS

XG drafted the full manuscript. JYZ and JWL was mainly contributed to edit the manuscript.

CONFLICTS OF INTEREST

The authors have none to declare.

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